AD_____

GRANT NO: DAMD17-94-J-4343

TITLE: Breast Cancer Tissue Repository

PRINCIPAL INVESTIGATOR(S): J. Dirk Iglehart, M.D.

CONTRACTING ORGANIZATION: Duke University Medical Center
Durham, North Carolina 27710

REPORT DATE: September 1995

TYPE OF REPORT: Annual

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. AGENCY USE ONLY *(Leave blank)*	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	September 1995	Annual 1 Sep 94 - 31 Aug 95

4. TITLE AND SUBTITLE
Breast Cancer Tissue Repository

5. FUNDING NUMBERS
DAMD17-94-J-4343

6. AUTHOR(S)
J. Dirk Inglehart, M.D.

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Duke University Medical Center
Durham, North Carolina 27710

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for public release; distribution unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT *(Maximum 200 words)*

During the preceding year, the Duke Breast Cancer Tissue Repository collected breast cancer tissue from 124 patients undergoing surgery for primary breast cancer at Duke Hospital. In 119 patients, tumor tissue was also embedded in gelatin for special applications. In most patients, matched normal or metastatic tissue was collected. In all, more than 1700 pieces of tissue were inventoried and frozen. Blood (serum, plasma, and WBCs) were collected from 145 patients. During the past year, more than 1000 pieces of tissue were distributed to more than 12 laboratories at Duke and in other institutions, private and public. During the upcoming year, the Repository will provide tissue for a newly funded Specialized Program of Research Excellence (SPORE) in Breast Cancer at Duke. The Repository will support an expanding program of molecular genetics at Duke by assisting in family ascertainment and blood procurement.

14. SUBJECT TERMS
Breast Tissue, Tissue Repository, Tissue Bank. Serum Bank, Serum Repository

15. NUMBER OF PAGES
8

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	Unlimited

GENERAL INSTRUCTIONS FOR COMPLETING SF 298

The Report Documentation Page (RDP) is used in announcing and cataloging reports. It is important that this information be consistent with the rest of the report, particularly the cover and title page. Instructions for filling in each block of the form follow. It is important to ***stay within the lines*** to meet ***optical scanning requirements***.

Block 1. Agency Use Only *(Leave blank)*.

Block 2. Report Date. Full publication date including day, month, and year, if available (e.g. 1 Jan 88). Must cite at least the year.

Block 3. Type of Report and Dates Covered. State whether report is interim, final, etc. If applicable, enter inclusive report dates (e.g. 10 Jun 87 - 30 Jun 88).

Block 4. Title and Subtitle. A title is taken from the part of the report that provides the most meaningful and complete information. When a report is prepared in more than one volume, repeat the primary title, add volume number, and include subtitle for the specific volume. On classified documents enter the title classification in parentheses.

Block 5. Funding Numbers. To include contract and grant numbers; may include program element number(s), project number(s), task number(s), and work unit number(s). Use the following labels:

C	- Contract	**PR**	- Project
G	- Grant	**TA**	- Task
PE	- Program Element	**WU**	- Work Unit Accession No.

Block 6. Author(s). Name(s) of person(s) responsible for writing the report, performing the research, or credited with the content of the report. If editor or compiler, this should follow the name(s).

Block 7. Performing Organization Name(s) and Address(es). Self-explanatory.

Block 8. Performing Organization Report Number. Enter the unique alphanumeric report number(s) assigned by the organization performing the report.

Block 9. Sponsoring/Monitoring Agency Name(s) and Address(es). Self-explanatory.

Block 10. Sponsoring/Monitoring Agency Report Number. *(If known)*

Block 11. Supplementary Notes. Enter information not included elsewhere such as: Prepared in cooperation with...; Trans. of...; To be published in.... When a report is revised, include a statement whether the new report supersedes or supplements the older report.

Block 12a. Distribution/Availability Statement. Denotes public availability or limitations. Cite any availability to the public. Enter additional limitations or special markings in all capitals (e.g. NOFORN, REL, ITAR).

- **DOD** - See DoDD 5230.24, "Distribution Statements on Technical Documents."
- **DOE** - See authorities.
- **NASA** - See Handbook NHB 2200.2.
- **NTIS** - Leave blank.

Block 12b. Distribution Code.

- **DOD** - Leave blank.
- **DOE** - Enter DOE distribution categories from the Standard Distribution for Unclassified Scientific and Technical Reports.
- **NASA** - Leave blank.
- **NTIS** - Leave blank.

Block 13. Abstract. Include a brief *(Maximum 200 words)* factual summary of the most significant information contained in the report.

Block 14. Subject Terms. Keywords or phrases identifying major subjects in the report.

Block 15. Number of Pages. Enter the total number of pages.

Block 16. Price Code. Enter appropriate price code *(NTIS only)*.

Blocks 17. - 19. Security Classifications. Self-explanatory. Enter U.S. Security Classification in accordance with U.S. Security Regulations (i.e., UNCLASSIFIED). If form contains classified information, stamp classification on the top and bottom of the page.

Block 20. Limitation of Abstract. This block must be completed to assign a limitation to the abstract. Enter either UL (unlimited) or SAR (same as report). An entry in this block is necessary if the abstract is to be limited. If blank, the abstract is assumed to be unlimited.

*U.S.GPO:1993-0-358-779

FOREWORD

Opinions, interpretations, conclusions and recommendations are those of the author and are not necessarily endorsed by the US Army.

NO Where copyrighted material is quoted, permission has been obtained to use such material.

NO Where material from documents designated for limited distribution is quoted, permission has been obtained to use the material.

✓ Citations of commercial organizations and trade names in this report do not constitute an official Department of Army endorsement or approval of the products or services of these organizations.

✓ In conducting research using animals, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Resources, National Research Council (NIH Publication No. 86-23, Revised 1985).

YES For the protection of human subjects, the investigator(s) adhered to policies of applicable Federal Law 45 CFR 46.

N/A In conducting research utilizing recombinant DNA technology, the investigator(s) adhered to current guidelines promulgated by the National Institutes of Health.

N/A In the conduct of research utilizing recombinant DNA, the investigator(s) adhered to the NIH Guidelines for Research Involving Recombinant DNA Molecules.

YES In the conduct of research involving hazardous organisms, the investigator(s) adhered to the CDC-NIH Guide for Biosafety in Microbiological and Biomedical Laboratories.

Accesion For	
NTIS CRA&I	☑
DTIC TAB	☐
Unannounced	☐
Justification	
By	
Distribution /	
Availability Codes	
Dist	Avail and / or Special
A-1	

PI - Signature Date

9/16/98

Table of Contents
DAMD17-94-J-4343
Breast Cancer Tissue Repository

Statement of Purpose

The purpose of the Breast Cancer Tissue Respository at Duke University is to provide a resource of tissue from breast cancer patients, patients with benign breast diseases, and normal clinical material from women undergoing cosmetic surgery. The Repository serves investigators at Duke University and collaborators at other private and public institutions. The Repository is intented for high-volume use and is not used as an archive of clinical material. We specialize in custom tissues for molecular studies, including flash-frozen tumor tissue, tissue embedded immediately in gelatin, matched sets of normal tissue and metastatic tumor sites to compliment the primary tissue. The Repository will collect material in a tailor-made fashion for special applications of primarily Duke investigators. Serum samples are collected on nearly all patients with breast abnormalities who are undergoing biopsy; follow-up samples are obtained from cancer patients during follow-up.

Introduction

During the past year, the Breast Cancer Tissue Repository at Duke University has grown substantially and has been well used. The object of this tissue bank is to create a resource for investigators at Duke and for collaborators at other institutions. It has been our intent to foster a high turn-over of specimens in the bank and discourage maintainence of a large inventory. As such, this resource is not used for long-term prognostic type studies but emphasizes short-term biological questions. In addition, the presence of dedicated personnel allows us to offer investigators tailored tissue collection, as outlined below. During the past year, investigators at Duke competed for an NIH Specialized Program of Research Excellence (SPORE) in Breast Cancer. We were successful in this competition and our SPORE will begin on October 1, 1995. The existence of the Department of the Army tissue repository assisted us in securing a SPORE and the Repository will now serve the entire SPORE at Duke and has been publicized among the other SPORE institutions. In addition, the SPORE has added to the bank in complimentary way, outlined below.

Body

Inventory Summary

As of September, 1995 the Repository has collected samples from 828 patients. This includes samples from 704 patients banked since 1988 and an additional 124 patients banked this year with support from the Department of the Army. The total number of frozen vials in our inventory is 5557, including 1776 vials banked during the past year of funding. The total number of tissue pieces numbers in the 10's of thousands.

This year we added routine embedding of tissue in gelatin (OCT) medium for in-situ applications. We collected gelatin-embedded blocks from 119 patients with breast cancer. In addition, we added routine pre- and post-operative blood draws from all

patients undergoing breast operations. We have collected blood from 145 patients from which we have stored WBC pellets separately from plasma and serum samples. The following table displays the histologic diagnosis of the tissues banked during the past year.

Table 1. Histology of samples collected between 9-1-94 and 9-1-95

Histology	Number acquired
Normal	19
Invasive ductal (NOS)	60
Invasive lobular	9
Specialized histologies (eg, medullary,etc)	3
Benign (eg, fibroadenoma)	1
Pure noninvasive (DCIS)	9
miscellaneous (recurrent, etc)	13

Summary of Usage

Usage of the bank has been high during the past year. Overall, more than 2000 samples have been distributed to investigators. It is important to emphasize that these investigators are from departments throughout the medical center and at collaborating institutions. The following table will summarize the investigators receiving material, the amount of material provided, and their departmental affiliations.

Table 2. Summary of usage between 9-1-94 to 9-1-95.

Investigator	Affiliation	Number of samples provided
Dr. Jeff Marks	Surgery, Duke	800
Dr. Andy Futreal	Surgery, Duke	30
Dr. Kevin Peters	Medicine, Duke	40
Dr. Chuck Greenberg	Medicine, Duke	116
Dr. Nick Bandarenko	Pathology, Duke	272
Dr. Kim Lyerly	Surgery, Duke	12
Dr. Randy Jirtle	Radiation Oncol., Duke	100
Dr. Carl Barrett	NIEHS	50
Dr. Lee Babbiss	Glaxo, Inc.	16
Dr. Laura Hale	Pathology, Duke	35
Dr. Joe Nevins	Genetics, Duke	194
Dr. Andrew Berchuck	Ob-Gyn, Duke	50

Specialized collection was done during the past year for Dr. Kent Weinhold in the Departments of Surgery and Immunology. Dr. Weinhold was provided fresh tumor specimens in order to proliferate tumor infiltrating lymphocytes and study their behavior.

In addition, he was provided with freshly collected heparinized blood in order to immortalize peripheral B-cells. In total, we provided him with 38 fresh tumor samples and heparinized blood from 41 patients. We provided Dr. Kim Lyerly in the Department of Surgery and the Program of Molecular Therapeutics within the medical center with 11 samples of fresh tumor. This material was used to isolate breast cancer cells and infect these cells with vectors expressing cytokine genes. For Dr. Randy Jirtle, we provided plasma freshly collected and frozen for analysis of TGF-beta determination from 136 patients. For Dr. Andy Futreal, we provided blood samples and WBC's from 41 patients with strong family histories of breast cancer. Finally, for Dr. Andrew Berchuck, we provided 75 WBC samples for testing methods of BRCA1 determination. Each of these requests was prospective and required special procedures to accomplish.

Expanding Roles for the Department of Army Respository at Duke

Last year at this time, the first breast cancer susceptibility gene was cloned by collaborating groups at Duke, the NIEHS, Utah, and Sloan-Kettering. This gene, BRCA1, was isolated by Dr. Andy Futreal working with Dr. Roger Wiseman at the NIEHS. During previous years, much material from Duke was sent to Drs. Futreal and Wiseman for this effort. In February, 1995 we recruited Andy Futreal to Duke to lead our molecular genetic research. To this end, the Repository was called upon to perform family ascertainment and blood collection. To date, the Repository has assisted in the ascertainment of more than 50 large kindreds with an excess of either breast or breast and ovarian cancer. Blood acquisition has begun from selected families and will continue to require effort during the next year. This material will be used by an international consortium of investigators to identify BRCA2, the second breast cancer susceptibility gene. In addition, investigators within the Duke SPORE in Breast Cancer have protocols underwhich gene testing will be studied in clinical practice. The Repository will provide venapuncture and storage services to these investigators as we are able. This activity is a perfect application for the Department of the Army Repository and provides material to a large number of investigators both at Duke and beyond.

Relation of the Army Repository to the Duke SPORE in Breast Cancer

The NIH requires that each SPORE in Breast Cancer include a Tissue Repository which must be a line-item in each SPORE budget. We proposed an addition to the Army Repository in our SPORE and cited the current repository as a mechanism to provide tissue to a larger group of investigators. The additional features of the SPORE Repository are as follows: 1.) provision of established cell-lines to investigators, 2.) attempt to culture breast cancers in-vitro and in immune-compromised animals, 3.) provision of animals bearing human tumor grafts from established cell-lines, and 4.) provision of a specialized window-chamber preparation to investigators studying aspects of breast tumor physiology (eg, angiogenesis, macromolecule delivery, etc). The SPORE provides an additional $45,000 per year to accomplish these efforts, which are non-overlapping and complimentary. The SPORE funding will last for three years and will

overlap exactly with funding from the Army. Dr. Iglehart is the Principal Investigator for the SPORE Core Tissue Resource, Director of the Duke SPORE, and Principal Investigator for the Army Repository.

Conclusions

The Department of the Army Breast Cancer Tissue Repository at Duke University is a high-volume resource which has served investigators in a variety of Departments and Programs within Duke Medical Center. In future years, the Repository will serve the Duke SPORE in Breast Cancer and will be made available to the other five SPORE programs. Our expanding roles will include assisting Duke investigators and their collaborators in studying the molecular genetics of breast cancer and in performing clinical research concerned with genetic susceptibility testing. The Army Repository will interdigitate with the SPORE Core Tissue Resource by performing different and complementary functions.